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Phenotypic Classification of Male Pseudohermaphroditism Due to Steroid 5α-Reductase 2 Deficiency

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Conversion of testosterone (T) to dihydrotestosterone (DHT) in genital tissue is catalysed by the enzyme 5α-reductase 2, which is encoded by the SRD5A2 gene. The potent androgen DHT is required for full masculinization of the external genitalia. Mutations of the SRD5A2 gene inhibit enzyme activity, diminish DHT formation, and hence cause masculinization defects of varying degree. The classical syndrome, formerly described as pseudovaginal perineoscrotal hypospadias, is characterized by a predominantly female phenotype at birth and significant virilization without gynecomastia at puberty.

We investigated nine patients with steroid 5α-reductase 2 deficiency (SRD). Phenotypes, which were classified according to the severity of the masculinization defect, varied between completely female (SRD type 5), predominantly female (SRD type 4), ambiguous (SRD type 3), predominantly male with micropenis and hypospadias (SRD type 2), and completely male without overt signs of undermasculinization (SRD type 1). T/DHT-ratios were highly increased (>50) in the classical syndrome (SRD type 5), but variable in the less severe affected patients (SRD types 1–4) (14–35). Mutations in the SRD5A2 gene had been characterized using PCR-SSCP analysis and direct DNA sequencing. A small deletion was encountered in two patients, while all other patients had single base mutations which result in amino acid substitutions.

We conclude that phenotypes may vary widely in patients with SRD5A2 gene mutations spanning the whole range from completely female to normal male without distinctive clinical signs of the disease. Hence, steroid 5α-reductase deficiency should be considered not only in sex reversed patients with female or ambiguous phenotypes, but also in those with mild symptoms of undermasculinization as encountered in patients with hypospadias and/or micropenis. A classification based on the severity of the masculinization defect may be used for correlation of phenotypes with enzyme activities and genotypes, and for comparisons of phenotypes between different patients as the basis for clinical decisions to be made in patients with pseudohermaphroditism due to steroid 5α-reductase 2 deficiency. © 1996 Wiley-Liss, Inc.

KEY WORDS: SRD5A2, 5α-reductase 2 deficiency, phenotype, classification, male pseudohermaphroditism, testosterone, dihydrotestosterone

INTRODUCTION

Conversion of testosterone (T) to dihydrotestosterone (DHT) is catalysed by the 5α-reductase enzymes. Two genes have been cloned for the two isoenzymes of 5α-reductase, which are rather homologous [Andersson

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

and Russell, 1990; Jenkins et al., 1991, 1992; Labrie et al., 1992]. However, their biochemical characteristics and their expression in different tissues differ substantially [Andersson and Russell, 1990; Thigpen et al., 1992; Wilson et al., 1993]. The steroid 5α-reductase type 2 is preferentially expressed in genital tissue and hence, is responsible for male sexual development. The SRD5A2 gene is located on chromosome 2, its coding region consists of five exons and translates into a protein of 254 amino acids [Wilson et al., 1993]. The conversion product DHT, which is a potent androgen, is required for full masculinization of the external genitalia. Mutations of the SRD5A2 gene inhibit enzyme activity, diminish DHT formation, and cause masculinization defects of varying degree [Wilson et al., 1993]. In the classical syndrome, the external genitalia appear completely female at birth and significant virilization without gynecomastia occurs at puberty. However, less severe cases with ambiguous or predominantly male genitalia have also been reported [Carpenter et al., 1990; Ng et al., 1990; Hiort et al., 1996].

The differentiation of steroid 5α-reductase 2 deficiency (SRD) from other causes of male pseudohermaphroditism can be difficult in prepubertal patients. Measurements of serum T and DHT concentrations after hCG stimulation and calculation of T/DHT ratios, and enzyme activity measured in genital skin fibroblasts have been used for the diagnosis of 5α-reductase deficiency. Whilst the classical syndrome with severely inhibited enzyme function can easily be discovered by these parameters, mild forms of enzyme deficiency may be difficult to detect.

On the basis of nine patients with steroid 5α-reductase 2 deficiency, whose diagnoses were established by means of hormone measurements in serum and molecular genetic analysis of the SRD5A2 gene, we propose a phenotypic classification system for the quantification of the severity of the masculinization defect in patients with male pseudohermaphroditism due to steroid 5α-reductase 2 deficiency.

MATERIALS AND METHODS

Hormone Measurements

Testosterone (T) and dihydrotestosterone (DHT) were measured by RIA using rabbit polyclonal antisera against testosterone-3-o-carboxymethyloxime bovine serum albumin conjugate which exhibit low (<40%) and high (>95%) crossreactivity to DHT, respectively. [^{3}H]-labeled T and DHT were used as tracers and dextran-coated charcoal as the separation agent. Prior to radioimmunoassay, serum samples were extracted in duplicate with ether. For DHT analysis, the extracts were further purified by column chromatography on Celite with isooctane followed by isooctane-ethyl acetate (95:5 vol/vol) as mobile phases in order to separate DIIT from T. Procedural losses as controlled by recovery of [^{3}H]-labeled as well as unlabeled T and DHT added to a male serum pool amounted to about 10% (T), and 40% (DHT), respectively. They were taken into account only for the calculation of DHT concentrations. With quality control samples representing normal male concentrations, interassay variances were 10% for T and 16% for DHT.

HCG Stimulation Tests

In prepubertal patients T and DHT measurements were performed after gonadal stimulation with human chorionic gonadotropin (hCG); 5,000 IU hCG per m^2 body surface area were injected i.m., blood was drawn once before and 3 days after the injection. If the T value did not rise above 10 nmol/L, a second protocol was used, which consisted of seven injections of 1,500 IU hCG every other day (adapted from Saez and Bertrand, 1968). Blood was drawn once before the first and one day after the last injection.

DNA-Analysis

DNA analysis was performed as described in detail elsewhere [Hiort O, et al., 1996]. Briefly, DNA was extracted from EDTA-blood. Exon 1 to 5 of the SRD5A2 gene were amplified by PCR. The amplification products were screened for variations by non-radioactive single strand conformation polymorphism (SSCP) analysis [Hiort O, et al., 1996]: Non-isotopic single strand confirmation analysis of the 5α-reductase type 2 gene for the diagnosis of 5α-reductase deficiency (submitted). The samples showing aberrant migration on SSCP analysis were sequenced.

Patients

Nine patients with steroid 5α-reductase 2 deficiency were studied. They were referred by physicians cooperating in the German Collaborative Intersex Study. All had a normal male karyotype 46,XY. Except for patients 8 and 9, who are brothers, all other patients are not related. However, the parents of patients 3–7 are consanguineous. In patients with ambiguous genitalia diagnostic work-up had been initiated in infancy; however, the diagnosis of 5α-reductase 2 deficiency was completed at various ages between 5 months and 22 2/12 years. In order to exclude androgen insensitivity, molecular studies of the androgen receptor (AR) gene [Hiort et al., 1993, 1994a,b] were performed in all patients and an SHBG androgen sensitivity test [Sinnecker and Köhler, 1989] was performed in patients 5, 6, 8, and 9. The results of these investigations were normal.

Patient 1 (referring pediatricians Dr. Hoepffner, Leipzig; Dr. Quietzsch, Plauen) (German, parents not consanguineous, no other relatives affected) presented at age 4 months with a normal female phenotype (Fig. 1e). Gonads of 13 mm length were palpable in the labia majora, no uterus was detectable by ultrasonography and magnetic resonance tomography. Serum LH (2.6 IU/L) and FSH (1.5 IU/L) were normal, testosterone increased from 5.2 nmol/L to 10.8 nmol/L after hCG stimulation. DHT increased to 0.18 nmol/L. The T/DHT-ratio was consecutively increased to 61, suggesting steroid 5α-reductase deficiency. Diagnosis was confirmed by molecular genetic characterization of two different heterozygous mutations (Ile_{112}-Asn, Glu_{126}-Arg) in exon 2 of the SRD5A2 gene (Table I).

Patient 2 (referring geneticists: Prof. Gal, Hamburg, Dr. Hauß, Oberhausen) (German, not consanguineous,

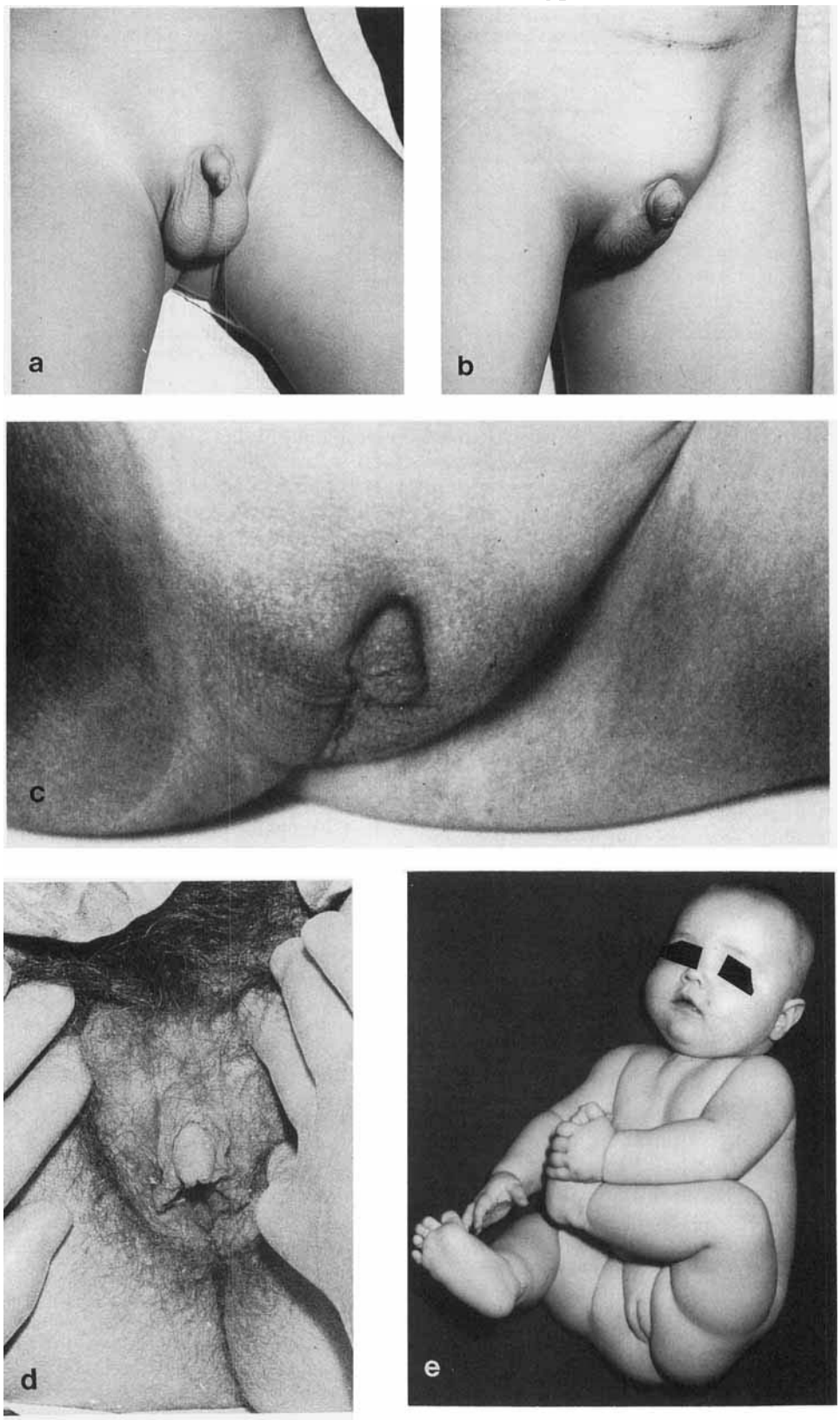

Fig. 1. Classification of phenotypes in male pseudohermaphroditism due to steroid 5α-reductase deficiency (SRD). **a:** A patient representing SRD type 1, which is anatomically normal male. **b:** SRD type 2, which is predominantly male with either isolated hypospadias, or micropenis, bifid scrotum, and severe hypospadias. **c:** SRD type 3 (ambiguous genitalia). **d:** SRD type 4 which is predominantly female with clitoral enlargement. **e:** SRD type 5 which is completely female (for details see Table II).

TABLE I. Age, Phenotype, SRD5A2 Gene Mutation, and Androgen Values in Nine Patients With Male Pseudohermaphroditism Due to Steroid 5α-Reductase 2 Deficiency*

Pat	Age (yr;mo)	SRD type	Gender	Amino acid change	Testo nmol/L	DHT nmol/L	T/DHT
1	0;5	5	f	Ile_{112}-Asn Gln_{126}-Arg	10.8	0.18	61
2	22;2	4b	f	His_{231}-Arg	Norm.	n.d.	n.d.
3	14;0	4a	f	Δ Met_{157}	19.3	0.67	28
4	14;3	3b	f	Δ Met_{157}	2.6	0.15	17
5	3;10	3a	m	Ala_{228}-Thr	13.5	0.83	16
6	10;10	2b	m	Gly_{196}-Ser	22.5	1.28	18
7	1;0	2b	m	Gly_{196}-Ser	11.8	0.87	14
8	7;10	2b	m	Arg_{227}-Gln	17.7	0.52	35
9	4;1	1a	m	Arg_{227}-Gln	25.7	0.80	32

*Gender, assigned gender; testo, testosterone; DHT, dihydrotestosterone; norm., value within the normal male range; n.d., not determined.

identical twin sister also affected) was born with predominantly female genitalia; however, the clitoris was significantly enlarged and openings of urethra and vagina were separated. Gonads were palpable in the labia majora. At the age of 12 years the voice broke and further clitoral enlargement was observed (Fig. 1d) which caused her and her identical twin sister who was also affected, to seek medical advice. Serum T was in the normal male range, DHT was not determined. At laparoscopy no uterus was identified, gonads were removed, which appeared histologically as normal testes. DNA analysis demonstrated a homozygous point mutation (His_{231}-Arg) in the SRD5A2 gene.

Patient 3 (referring pediatrician: Prof. Heinrich, Heidelberg) (Turkish, parents consanguineous) presented postnatally with a predominantly female phenotype. The clitoris was slightly enlarged, genitography demonstrated an urogenital sinus and a blind-ending vagina. Gonads were in the inguinal region. At the age of 14 years the clitoris was enlarged to 3 cm. After hCG stimulation serum T increased normally from 0.1 nmol/L to 19.3 nmol/L, whereas DHT increased relatively less, from <0.3 nmol/L to 0.67 nmol/L, resulting in an increased T/DHT-ratio of 28. Diagnosis was confirmed by means of DNA analysis (Δ Met_{157}) after gonadectomy had been performed.

Patient 4 (referring pediatrician: PD Dr. Dörr, Erlangen) (Turkish, parents consanguineous) had ambiguous genitalia at birth with clitorial enlargement, urogenital sinus, and marked labial fusion. Gonads were located in the inguinal region. She was assigned a female gender. In infancy she had had reconstructive genital surgery and removal of one gonad. At the age of $14\,^{3}/_{12}$ years she presented with signs of progressive virilization. Serum T was within the normal male range, DHT was relatively low, resulting in a slightly increased T/DHT-ratio (see Table I). Molecular genetic analysis documented the same homozygous mutation (Δ Met_{157}) as in patient 3.

Patient 5 (referring pediatricians: Dr. Hecker and Dr. Holder, Stuttgart) (Eritrean, the grandfathers were brothers). He presented at birth with ambiguous genitalia, perineal hypospadias, a hypoplastic scrotum with both gonads palpable in the inguinal canal, and a micropenis (Fig. 1c). On ultrasonography no uterus was seen. At age $3\,^{10}/_{12}$ years a prolonged hCG stimulation test according to protocol II showed a T/DHT-ratio of 16 (Table I). The diagnosis was established by means of the DNA analysis which showed a homozygous point mutation in the SRD5A2 gene (Table I). A clinical trial of 25 mg testosterone enanthate i.m. three times in 4 week intervals was initiated at the age of 8 months which resulted in an increase of penis size from 1.2 cm to 2.8 cm. For surgical repairs, operation of the undescended testes at the age of $2\,^{2}/_{12}$ years and then correction of the hypospadias at the age of $3\,^{10}/_{12}$ years were performed.

Patient 6 (referring pediatric surgeon: Dr. Hemminghaus, Herne; pediatrician: Dr. Pingel, Paderborn) (Turkish, parents consanguineous) presented at the age of 2 months with a predominantly male phenotype (penoscrotal hypospadias, chordee, micropenis of 1 cm, and bifid scrotum). Genitography and genitoscopy showed no vaginal rudiment. Prolonged hCG stimulation (protocol II) resulted in a slightly increased T/DHT ratio of 18. DNA analysis showed a homozygous point mutation of the SRD5A2 gene, leading to a substitution of glycine by serine in position 196 [Hiort et al., 1996].

Patient 7 (referring pediatrician: Dr. Schnabel, Berlin) (Turkish, parents are first cousins) was found at birth to have a bifid scrotum, descended testes, perineal hypospadias, and a micropenis of 1 cm with chordee. Genitography gave no evidence of Müllerian structures. Hormone values were within the normal ranges for age, the T/DHT-ratio did not exceed the normal range after hCG stimulation (for details, see Table I). On molecular genetic analysis the same homozygous point mutation was found as in patient 6 (Gly_{196}-Ser).

Patients 8 and 9 are brothers of Vietnamese origin (referring pediatrician: Dr. Albers, Hannover). The older brother (patient 8) was $7\,^{10}/_{12}$ years old at initial presentation for endocrine evaluation. He had scrotal hypospadias with a bifid scrotum, and a small (3 cm) penis (Fig. 1b). The testes had descended spontaneously at the age of $3\,^{11}/_{12}$ years, repair of hypospadias was initiated as a staged procedure during the first years of life. His younger brother (patient 9, $4\,^{1}/_{12}$ years old) had a small, albeit normal penis of 2.5 cm length. No overt sign of defective masculinization was present

(Fig. 1a). Müllerian remnants were not detectable in either child by ultrasonography. Prolonged hCG stimulation resulted in significantly increased T/DHT ratios of 35 and 32, respectively. DNA analysis showed the same homozygous point mutation in both brothers, causing arginine to be replaced by glutamine in position 227 of the SRD5A2 gene [Hiort et al., 1996].

Statistics

Statistical analysis was performed using SPSS/PC+ (Chicago, IL) statistical software. Mann-Whitney *U* test and Pearson's correlation coefficient were performed as appropriate.

RESULTS

Based on the diverse phenotypes observed in our patients and on those described in the literature [Wilson et al., 1993], we defined a classification system for the quantification of the severity of the masculinization defect in patients with steroid 5α-reductase 2 deficiency (SRD). This classification is based on the clinical categories of steroid 5α-reductase deficiency: a minimal form without overt signs of undermasculinization (SRD type 1), a partial form with predominantly male phenotype (SRD type 2), a partial form with frankly ambiguous genitalia (SRD, type 3), a partial form with predominantly female phenotype (SRD type 4), and the complete syndrome with female external genitalia (SRD type 5) (Table II) (Fig. 2).

According to these categories we classified our patients as follows: Patient 1, whose phenotype was completely female without any signs of androgen effects, was most severely affected from steroid 5α-reductase deficiency. This complete masculinization defect was designated as SRD type 5 (Fig. 1e). Patient 2, in whom slight signs of virilization were observed, was classified as SRD type 4. Since urethral and vaginal openings were distinct (no urogenital sinus), patient 2 was subclassified as SRD type 4b (Fig. 1d). In contrast, patient 3 was classified as SRD type 4a, since her genitalia, although predominantly female as well, had an urogenital sinus. Patient 4, although carrying the same mutation as patient 3, had ambiguous genitalia and an urogenital sinus and was classified as SRD type 3b. Patient 5, who had ambiguous genitalia as well, was classified as SRD type 3a, since no urogenital sinus and no vagina were present. Patients 6,. 7, and 8 had micropenis and hypospadias. They were classified as SRD type 2b. In contrast, patient 9, who had no overt signs of undermasculinization was classified as SRD type 1 (Fig. 1a).

No significant correlation was found between the degree of undermasculinization (SRD phenotype) and the patient's T/DHT-ratio in serum.

DISCUSSION

Steroid 5α-reductase 2 deficiency leads to incomplete masculinization of individuals with a normal male karyotype. In severely affected adolescents or adults the sex reversed phenotype, pubertal virilization without gynecomastia, high serum testosterone levels and increased T/DHT-ratios allow the diagnosis to be made easily. During infancy and childhood, diagnosis can be difficult in particular in less severely affected patients who present only with hypospadias and/or micropenis. However, clinical decisions about gender assignment, gonadectomy, and androgen treatment for microphallus have to be made early in life. Such decisions should be based on an accurate diagnosis which allows a prognosis about testicular function, sensitivity of genital tissues to the action of androgens, and hence, in addition to the phenotype at birth, allows a prognosis about the potential for pubertal virilization.

We have classified our patients according to the severity of the masculinization defect into five different categories (SRD types 1–5). This classification may be used to easily compare phenotypes between different patients and to correlate phenotypes with enzyme activities which may be assessed either in cultured genital skin fibroblasts or by determination of the T/DHT-ratio in serum.

Our patient 1 was classified as SRD type 5, according to the completely abolished DHT effect on the genitalia, indicated by the female phenotype. The Gln_{126}-Arg mutation that this patient carries, was described earlier in a different patient and severely impairs enzyme function [Thigpen et al., 1992]. The other mutation (Ile_{112}-Asn) induces also a non-conservative amino acid substitution and hence must be assumed to inhibit steroid 5α-reductase 2 activity (Hiort et al., submitted). However, although the T/DHT-ratio after hCG stimulation

TABLE II. Classification of Phenotypes in Male Pseudohermaphroditism Due to Steroid 5α-Reductase 2 Deficiency (SRD) (See Also Fig. 2)

Type	Phenotype	Subtype	Phenotype/function
1	Male	1a	No overt undermasculinization
		1b	Impaired spermatogenesis
2	Predominantly Male	2a	Isolated hypospadias
		2b	Micropenis and severe hypospadias, bifid scrotum
3	Ambiguous	3a	Microphallus with clitoris-like underdeveloped glans, labia majora like bifid scrotum, perineoscrotal hypospadias
		3b	As 3a but sinus urogenitalis with a short, blind ending vagina
4	Predominantly Female	4a	Clitoromegaly and labial fusion, sinus urogenitalis with a wide opening, short, blind ending vagina
		4b	Slight signs of androgen effects: slight clitoromegaly or partial labial fusion, distinct urethral and vaginal opening
5	Female	5	No signs of virilization (prepubertal or after gonadectomy)

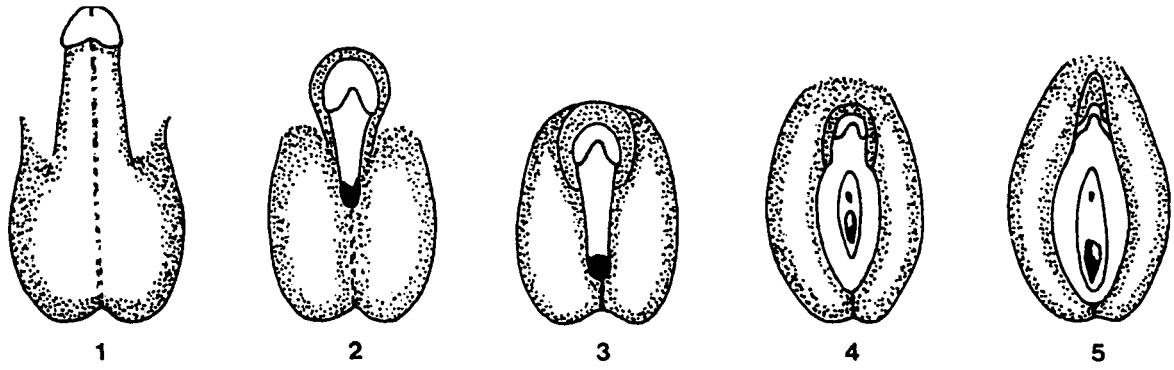

Fig. 2. Classification of phenotypes in male pseudohermaphroditism due to steroid 5α-reductase deficiency (SRD). 1, SRD type 1; 2, SRD type 2; 3, SRD type 3; 4, SRD type 4; 5, SRD type 5 (for details see Table II).

was highly increased in this patient, DHT was still measurable (Table I). As this is usually the case in patients with steroid 5α-reductase deficiency [Wilson et al., 1993], it indicates that 5α-reduction of testosterone is still present, even in severely affected patients. This is probably due to the activity of the isoenzyme 5α-reductase type 1, which contributes to the serum DHT concentration and may be responsible for the pubertal virilization which is regularly observed in such patients [Imperato-McGinley et al., 1979]. Since the phenotype of patients with SRD type 5 is female, they are to be assigned a female gender. However, the gonads have to be removed before puberty, to avoid the risk of pubertal virilization.

Patient 2 (SRD type 4b) had been assigned female. Unfortunately, the gonads had not been removed before puberty. Thus, she developed severe pubertal virilization. The homozygote His_{231}-Arg mutation she carries, has been reported in other patients of black and white American [Walsh et al., 1974; Thigpen et al., 1992] and of Polish [Boudon et al., 1995a] origin to severely inhibit enzyme activity [Thigpen et al., 1992]. However the American patients had more masculinized phenotypes, which could be classified as SRD type 3a. Nevertheless, all patients had been reared as females.

In patient 3 the T/DHT-ratio of 28 after hCG stimulation was significantly increased (normal ≤17 according to the data published by Pang et al., 1979 and Forest et al., 1990). Therefore the basal T/DHT-ratio of 17.3 in patient 4 can be considered borderline increased. Interestingly, although deletions are rarely observed in the SRD5A2 gene [Andersson et al., 1991] both, patients 3 and 4, had been found to carry the same homozygous deletion of 3 base pairs causing the methionine in position 157 of the enzyme protein to be lost. However, patient 4 had a different phenotype as the genitalia were ambiguous at birth with a short blind ending vagina. These phenotypic differences were addressed by the classification as SRD 4a for patient 3, while patient 4 was classified as SRD type 3b. The same mutation was reported recently in another Turkish patient who had clitoral hypertrophy, urogenital sinus, and a blind vaginal pouch [Boudon et al., 1995b].

Patient 5, who was classififed as SRD type 3a, had been assigned a male gender and was treated with testosterone as recommended by Burstein et al. [1979] for the treatment of microphallus. The T/DHT ratio of 16 was borderline, indicating that the T/DHT-ratio in serum does not discriminate well between normal and diminished 5α-reductase activity in genital tissue. Since mutations affecting exon 4 of the SRD5A2 gene, in particular those surrounding position 228 in which our patient carries a mutation, are known to inhibit NADPH binding and the binding capacity for testosterone [Thigpen et al., 1992; Wilson et al., 1993], a reduced enzyme activity due to this homozygote mutation can be assumed. Thus, in spite of almost normal T and DHT concentrations in serum, the genital malformation can be severe if 5α-reductase type 2 activity is diminished.

Patients 6 and 7 both were born with only slight signs of defective masculinization and were therefore designated males. Both carry a homozygous mutation (Gly_{196}-Ser) in the SRD5A2 gene which has also been reported in another patient with a similar phenotype of Greek origin [Carpenter et al., 1990; Thigpen et al., 1992]. Transfection studies have shown NADPH-binding to be primarily affected, resulting in diminished activity and half-life of the enzyme protein [Wigley et al., 1994]. The genital malformation in these patients can clearly be considered to be due to steroid 5α-reductase 2 deficiency. They were classified as SRD type 2b. However, the T/DHT-ratio was only slightly increased in patient 6, but was normal in patient 7. This indicates that a normal serum T/DHT-ratio does not exclude 5α-reductase deficiency.

The two brothers (patients 8 and 9) who carry the Arg_{227}-Gln mutation presented with different phenotypes. While the older one was classified as SRD type 2 (micropenis, hypospadias, and chordee), his brother had only a small, but normal penis. We have designated this clinical category of diminished 5α-reductase activity (as evidenced by an increased T/DHT-ratio) due to a SRD5A2 gene mutation, as SRD type 1. In spite of these phenotypic differences, T/DHT-ratios were not

different and highly increased in both, exceeding all but one other values measured in this study (Table I). This observation indicates that the T/DHT-ratio does not correlate with the severity of the masculinization defect. Accordingly, no correlation was found between the severity of the masculinization defect (*i.e.,* SRD phenotypes) and T/DHT-ratios.

The variability of phenotypes in patients who carry the same mutation may be due to variable hormone levels, androgen receptor activities and, in particular, to the activity of the 5α-reductase type 1 isoenzyme. In contrast to the data from the literature, in which less than 20% (7 out of 42 patients) of the patients with steroid 5α-reductase 2 deficiency are raised as males [Wilson et al., 1993], in our study five of nine had been assigned a male gender. This difference may be due to the fact that the more severely affected patients with the classical female phenotype have been preferentially diagnosed in the past when molecular genetic analysis had not been available. Owing to the variable hormone measurements which depend on sufficient gonadal stimulation, which is difficult to accomplish during infancy and childhood, most less severely affected patients may have been failed to be diagnosed in the past. However, since masculinization defects due to steroid 5α-reductase activity can well be treated with either systemic testosterone [Price et al., 1984] or topical DHT [Carpenter et al., 1990], it is prudent to consider this enzyme defect in the differential diagnosis of any genital malformation. If the diagnosis of steroid 5α-reductase deficiency has been established, it seems to be advisable to choose a male sex of rearing whenever possible (*i.e.,* at least in SRD types1–3). Short-term androgen treatment may facilitate surgical repair of hypospadias and may normalize phallic length [Burstein et al., 1979]. In severely affected patients (SRD types 4–5) a female gender may be assigned. In these cases early gonadectomy is prudent to avoid the risk of pubertal virilization.

We conclude that phenotypes may vary widely in patients with SRD5A2 gene mutations spanning the whole range from completely female to normal male without distinctive clinical signs of the disease. Hence, steroid 5α-reductase deficiency should be considered not only in sex reversed patients with female or ambiguous phenotypes, but also in those with mild symptoms of undermasculinization as encountered in patients with hypospadias and/or micropenis. The classification proposed in this study may be used for correlation of phenotypes with enzyme activities and genotypes, and for comparisons of phenotypes between different patients as the basis for clinical decisions to be made in patients with pseudohermaphroditism due to steroid 5α-reductase 2 deficiency.

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